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Modern biochemical methods used in the diagnosis of experimental toxic hepatitis

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Abstract: Oxygen is required by the cells of most organisms to produce sufficient ATP for metabolic activity. Hypoxia, or oxygen deprivation, occurs in human tissues and cells due to a variety of conditions, including heart and lung diseases, anemia, and circulatory disorders. Depending on the severity, irreversible tissue and cell damage may occur [1].
Keywords:

Oxygen is required by the cells of most organisms to produce sufficient ATP for metabolic activity. Hypoxia, or oxygen deprivation, occurs in human tissues and cells due to a variety of conditions, including heart and lung diseases, anemia, and circulatory disorders. Depending on the severity, irreversible tissue and cell damage may occur [1].

However, hypoxia can also play an important and beneficial role in human physiology and development. This is an integral part of proper embryonic development. Although the exact mechanisms are unknown, oxygen tension is associated with neural tube closure, mediating apoptosis and proper morphological development during pregnancy. Such evidence suggests that in addition to genetic signals, environmental conditions such as hypoxia serve as signals in embryonic development [2,3,4]

Many organisms have developed adaptation mechanisms to hypoxic conditions. Changes in oxygen levels can lead to the activation or repression of certain homeostatic regulatory genes, allowing tissues and cells to survive despite fluctuating environmental conditions. Genes such as HIF-1, which are upregulated by hypoxic conditions, can interact with enzymes and other transcription factors to control vascularization and tissue growth. While the microenvironment surrounding cancer tumors are extremely hypoxic, the spread of such masses is often made possible by the activation of HIF-1, which leads to increased angiogenesis and thus increased oxygen supply to the area [5,6].

Given its important function, manipulating HIF-1 activity in areas of ischemia and tumor masses has become a major focus of efforts to develop non-invasive , pharmaceutical treatment options for patients with cancer and heart disease. Although no such human protein has been scientifically regulated successfully, controlling HIF-1 activity is becoming increasingly feasible as details of its structure, function, and genetic pathway are elucidated.

HIF-1 is a heterodimeric transcription factor consisting of constitutively expressed β- subunit and oxygen-regulated α-subunit. The HIF-1α and HIF-1β proteins both contain basic helix-loop-helix motifs that bind DNA and cause subunit dimerization [7,8,9]. Both subunits also have a Per -ARNT- Sim (PAS) domain with similar functions. The α subunit has an oxygen-dependent degradation domain (ODD) that is hydroxylated by proline hydroxylase-2 (PHD-2), making the α subunit vulnerable to proteasomal degradation under normoxic cellular conditions [10].

Transactivation domains (TADs) that regulate HIF-1 target genes. CREB binding protein (CBP) and p300, two transcriptional coactivators of HIF-1, interact with the carboxy-terminal transactivation domain (C-TAD) of HIF-1α.

Both activators are required for HIF-1 transcription and are therefore targets for the regulation of HIF-1 expression; inhibition of HIF-1α C-TAD interactions by hydroxylation proline suppresses the expression of the HIF-1 gene, preventing normal transcription and translation [11]. HIF-1β contains only one such analogous region, which is not required for complex HIF-1 function [7,10,12]. Recent reports indicate that HIF-1β is identical to a previously identified vertebrate protein, aryl hydrocarbon receptor nuclear translocator (ARNT).

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HIF-1 is a major regulator of oxygen homeostasis in cells. As a transcription factor, it influences and regulates the expression of dozens of genes involved in maintaining homeostasis when oxygen concentration changes [13]. HIF-1 further mediates cellular responses to hypoxia by regulating glucose uptake and anaerobic respiration in oxygen-depleted environments [5,2].)

One of the important functions of HIF-1 is to promote angiogenesis ; HIF-1 directs the migration of mature endothelial cells into a hypoxic environment [2,5]. This is accomplished through HIF-1 regulation of vascular endothelial growth factor (VEGF) transcription. VEGF is a master regulator of angiogenesis , which promotes the migration of endothelial cells towards the hypoxic region. During hypoxia, HIF-1 binds the regulatory region of the VEGF gene, inducing its transcription and initiating its expression [12,15,16]. These endothelial cells ultimately help form new blood vessels, supplying the area with oxygenated blood [14].

HIF-1 regulates the transition to anaerobic metabolism

HIF-1 may also regulate anaerobic metabolism. When oxygen is available, most cells produce ATP through oxidative phosphorylation . However, in a hypoxic environment there is a shift towards anaerobic metabolism to produce cellular energy. HIF-1 is one of the major genes that coordinates this shift, inducing various glycolytic enzymes and glucose transporters such as aldolase A and pyruvate kinase M, which help cells efficiently produce energy in hypoxic environments [5,16]. In addition to increasing the expression of these enzymes, HIF-1 reduces mitochondrial oxygen consumption by activating pyruvate dehydrogenase kinase I and stopping the citric acid cycle [17].

Cancer, inflammation and hypoxia

The environment surrounding metastatic tumor masses is often hypoxic. HIF-1 is the most important protein in such masses; it enables tumor progression by inducing alternative metabolic pathways in cancer cells, as discussed above in the context of physiological hypoxia.

Tumor spread

Due to its role in hypoxia, HIF-1 plays a crucial role in tumor proliferation [18]. As the tumor develops and grows, a hypoxic environment is created due to the extreme energy demands of numerous rapidly dividing cells. Such cell masses often induce angiogenesis to meet the demands for increased oxygen, energy and blood supply [5,16]. At the same time, HIF-1 promotes the transition to anaerobic metabolism. The importance of this transcription factor in tumor cell survival is reflected in the finding that HIF-1α levels in glioma tumor cells increase in proportion to tumor grade [19].

Mechanisms of HIF-1-mediated tumor survival partially revealed by Semenza et al . on VHL-deficient renal carcinoma cells. HIF-1 was found to reduce oxygen consumption in these cells by inhibiting C-MYC, a transcription factor that regulates mitochondrial mass and oxygen consumption and is known to be downregulated in various human cancers. SemeSemenza et al . report that HIF-1 reduces C-MYC levels by increasing transcription of MXI1, a C-MYC repressor, and by increasing the rate of proteasomal degradation of C-MYC protein. Reduced levels of C-MYC in these cancer cells were found to ultimately lead to increased glycolysis and decreased mitochondrial respiration, critical characteristics of cancer cells that survive and proliferate in the hypoxic conditions of the tumor microenvironment [30].

Overexpression of HIF-1 induces apoptosis

Currently, many studies are being conducted on the role of HIF-1 in hypoxia -induced apoptosis of various cell types. For example, Krick et al . recently reported that overexpression of HIF-1 in alveolar epithelial cells leads to increased apoptosis [21]. Although the exact pathways and mechanisms involved in this process remain unclear, evidence suggests that the tumor suppressor p53 is activated under hypoxic conditions. Through interaction with the HIF-1 protein, p53 is stabilized and begins to activate genes such as p21, which in turn cause cell death [5,21].

HIF-1 supports inflammatory responses and hypoxic recovery

In addition to other roles in adaptation to hypoxia, HIF-1 has been shown to play a role in inflammation. Kramer et al demonstrated that HIF-1 is required for metabolism in myeloid cells [22]. Overexpression of HIF-1 in vivo resulted in increased localized inflammation, while loss of the HIF-1 gene reduced the ability of myeloid cells to aggregate, migrate, and promote bactericidal responses. This dependence of myeloid cells on HIF-1 may be related to their dependence on anaerobic respiration as a means of energy production. Myeloid cells lacking this gene cannot efficiently produce ATP, migrate effectively into damaged tissues, or

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destroy foreign invaders [22]. In addition, HIF-1α expression plays a role in the differentiation of myeloid cells into monocytes and macrophages [23].

In contrast, HIF-1 may prevent tissue and heart damage caused by ischemia, which can lead to a variety of long-term heart problems. Overexpression of HIF-1 in such tissues can induce angiogenesis and thus increase oxygenation of the area [24,25]. This serves as the basis for ongoing efforts to find pharmaceutical and other noninvasive treatments for ischemia and related diseases.

Pathways of activation and suppression

Under normoxic conditions, HIF-1α is degraded by the proteasome . The HIF-1α subunit is "tagged" for such degradation by proline hydroxylase 2 (PHD-2) and von Hippel - Lindau (VHL) -ubiquitin ligase complexes . Consequently, HIF-1 does not function in the presence of sufficient oxygen [10,26]. Also, inactivation of HIF-1 under normoxic conditions is promoted by the protein inhibitory factor HIF-1 (FIH), which hydroxylates HIF-1, preventing the interaction of this subunit with the coactivators p300 and CBP. The expression and stabilization of the HIF-1 complex is regulated through feedback inhibition, as PHD-2 itself is activated by HIF-1 [12].

However, under hypoxic conditions, the HIF-1 protein is stable and active, since hydroxylases , VHL and FIH proteins are inhibited by lack of oxygen. HIF-1 can then interact with its coactivators and can dimerize with its constitutive expressed β- subunit. Once stabilized, the HIF-1 protein can bind to the regulatory regions of its target genes, inducing their expression [7,10,27].

Oxygen-independent stimuli

Different HIF-1 stimuli act independently of oxygen concentration. These stimuli are primarily proteins that regulate the translation of HIF-1, in sharp contrast to the hypoxic stimuli of this gene, which act on already expressed α- subunit. Protein kinase C (PKC) increases the transcription rate of HIF-1α and functions in concert with the phosphatidylinositol 3-kinase (PI3K) pathway, which also enhances HIF-1α translation. The PKC pathway activates the expression of ribosomal protein S6, which specifically recognizes transcripts mRNAs such as HIF-1α. By phosphorylating the S6 protein under normoxic conditions, the translation rate of HIF-1α mRNA can be significantly increased, effectively counteracting the effects of proteasomal degradation of this subunit and increasing cellular levels of the HIF-1 complex. The PI3K pathway has been identified as the primary means by which various mediators, such as lipopolysaccharides , influence HIF-1α activation in vascular smooth muscle cells and macrophages [12,27].

Therapeutic targets in the HIF-1 pathway: ischemia

Overexpression

When treating ischemia, activation of HIF-1α can stimulate angiogenesis and increase blood flow. Many genes involved in angiogenesis such as VEGF, matrix metalloproteinase 2 (MMP2), cathepsin D (CATHD) and keratin (KRT), are targets of the HIF-1 transcription complex. It is believed that increased levels of HIF-1 lead to a proportional increase in these proteins [12,28]. In several recent studies, mice injected with HIF-1α DNA without ODDD exhibited increased blood supply to wounded or ischemic areas, suggesting that increased HIF-1α levels may contribute to the supply of blood, oxygen and nutrients to areas of focal ischemia [29, 30].

Introduction of a constitutively stable HIF-1α hybrid into rat cardiomyocytes resulted in a decrease in ischemic damage. This hybrid consisted of DNA binding and dimerization domains from HIF-1α and the transactivation domain of the HSV VP16 protein [31]. Overexpression of HIF-1α in mouse models of myocardial infarction reduces infarct size, thereby preserving cardiac function [32]. Increasing HIF-1 expression may be a successful drug treatment for patients with ischemia who cannot undergo surgery.

Direct modifications of HIF-1

Direct Phosphorylation of the HIF-1α subunit may increase HIF-1 activity, presumably by interfering with proteasome /VHL recognition. Although very little is known about the phosphorylation of HIF-1α, the protein kinases activated mitogen p42/p44, phosphorylate this protein in vitro. In vivo, such phosphorylation is required for HIF-1 function. Activation of the p42/p44 pathway results in increased transcription levels of HIF-1α. This phosphorylation may be the optimal step in the HIF-1 pathway to induce overexpression [33].

hydroxylases are composed of several related molecules, including HIF inhibitory factor (FIH) proteins and prolyl hydroxylase domain (PHD) proteins. Because VHL mediates proteasomal degradation of hydroxylated HIF-1α, HIF-1α levels can be increased by inhibiting HIF-1alpha-prolyl-4 hydroxylase-2 (PHD2). Inhibition

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of PHD2 by siRNA also results in reduced myocardial infarct size in mice. These pathways can be modified using pharmacological approaches [34].

Small molecule inhibitors

Several small molecules, such as dimethyloxalylglycine , a prolyl hydroxylase inhibitor , activate HIF-1. Hydroxylase activity can be eliminated by mutating specific regions or by adding cobalt ions to the cell, which presumably compete for iron binding sites [35]. Some prolyl family hydroxylases can be selectively inhibited by adriamycin in vitro [36]. Cobalt(II) and nickel(II) ions in cells increase HIF-1 activity, presumably because such ions displace iron from the active sites of OG hydroxylases 2.

Small molecule therapy may be useful not only for suppressing HIF-1, but also for its activation in the treatment of ischemic diseases [7]. Hormones such as angiotensin II and platelet-derived growth factor stimulate the HIF pathway by increasing HIF-1α protein levels through the production of reactive oxygen species (ROS) in the cell. Although the exact mechanism is unclear, it appears to be completely different from the hypoxic pathways. Thrombin and other growth factors enhance angiogenesis through HIF-1α agonist mechanisms [14,33]. Insulin also activates HIF-1α, activating a variety of protein kinases required for expression and function [37].

In another HIF-1 activation study, homozygous deletion of the p53 gene led to activation of HIF-1 [38]. Therefore, p53, responsible for promoting ubiquitination of HIF-1α may be another possible target.

Ultimately, gene therapy may be used to increase HIF-1 levels and alleviate ischemic complications. For example, delivery of a stabilized recombinant form of HIF-1α via adeno-associated virus (AAV) to overexpress HIF-1 in skeletal muscle resulted in a significant increase in capillary number [38,39]. While gene therapy approaches targeting the process and effects of angiogenesis continue to be developed and studied, higher levels of success in preclinical trials are now being sought before clinical applications are pursued. One of the most notable remaining barriers to gene therapy is the mode of delivery [38]. The search for the most effective delivery vector continues.

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